

XLV. THE ANAEROBIC METABOLISM OF THE EARTHWORM (*LUMBRICUS TERRESTRIS*).

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INTRODUCTION.

THE investigation of the anaerobic metabolism of the earthworm was commenced simultaneously with that of the cockroach [Davis and Slater, 1926, 1928; Slater, 1927], and carried out in a similar manner.

Owing, however, to the greater variations which occurred in the experimental results obtained from the earthworm the investigation was for a time abandoned. It was recommenced in order to ascertain whether the apparently abnormal recovery process observed in the cockroach applied to other distantly related invertebrates.

In the case of the earthworm there is considerable evidence available as to the nature of the anaerobic metabolism [Lesser, 1907 *et seq.*]. Lesser, following the methods used by Weinland [1901, 1902] in the investigation of intestinal worms, obtained results from which he concluded that the energy for anaerobic life is supplied by the breakdown of glycogen to carbon dioxide, a fatty acid (probably valeric), and an unidentified product. More recent work has shown that Weinland was in error in the conclusions which he had drawn [Fischer, 1924; Slater, 1925], and hence doubt was also thrown on the analogous deductions made by Lesser in the case of the earthworm. It will be shown later that Lesser's results may be interpreted in support of the general view that the source of anaerobic energy is due to the breakdown of glycogen to lactic acid.

The methods employed in the investigation of the earthworm are almost identical with those used in the case of the cockroach, and in consequence details are given only when variations occur. Full descriptions will be found in the publications referred to above.

METHOD.

The determinations which have been made consist of the measurement of the oxygen intake at rest under normal conditions and following upon a period of anaerobiosis and the estimation of the lactic acid content before and after anaerobiosis.

In determining the oxygen intake certain factors had to be considered which did not present themselves in the case of the cockroach. It was necessary to decide whether the worms should be used with earth in the gut, or whether they should be kept under such conditions that before the experiment they would empty their guts completely. There were obvious objections to each procedure. Thus, if the guts contained earth, the weight of the earth could not be taken into account, and throughout the experiment a steady contractile movement of the gut wall occurred. On the other hand, if the guts were emptied before the experiment the animals appeared to behave abnormally, with varying activity of the gut muscle. After careful consideration it was decided that when the average of a number of determinations made under similar conditions was taken, the lesser error would occur when the normal worms with full guts were used.

The second difficulty arose from the sensitivity of the worms to direct light. It was found at an early stage of the work that direct light from the thermostat lamps caused the oxygen intake to rise to approximately double the value obtained in dull diffused light. The rise in metabolic rate was unaccompanied by any visible irritation. All the results given below have been obtained in the absence of any direct illumination.

In determining the lactic acid content it was not possible as in the case of the cockroach to remove the gut, so the whole animals were minced and extracted with a 4 % solution of trichloroacetic acid. It should be noted in this connection that the presence of the gut in the cockroach leads to high values for the resting lactic acid, and to irregular values after anaerobiosis; the average of the latter values, however, exceeds the corresponding average for the gutted insects by no more than the difference between control experiments on the ungutted and gutted insects.

EXPERIMENTAL RESULTS.

Resting metabolism. The normal resting oxygen intake was determined in order to ascertain the influence of size and other factors on the metabolic rate. As in the case of the cockroach, the larger animals showed a definitely lower oxygen intake. If any marked movement other than that of the gut occurred during the experiment, the result was rejected. In Table I are given the results for nine worms in order of increasing weight.

Table I. *Oxygen intake of Lumbricus terrestris at 25°.*

Body weight g.	Resting oxygen intake mm. ³ /g./hour
0.26	200
0.33	183
0.90	80
1.13	107
1.29	88
1.35	95
1.40	100
1.92	42
1.93	66

Anaerobic metabolism. Purified hydrogen was used to remove the air in the respiration chamber, and a slow stream maintained throughout the period of anaerobiosis.

As in the case of the cockroach the animals went into debt for oxygen during the anaerobic period and used an excess of oxygen afterwards to refund this deficit. The agreement was not good owing to the many variable factors, but the results of five typical experiments given in Table II show the general relationship between the oxygen of which the animal has been deprived and the excess which is used during recovery.

Table II. *Recovery of Lumbricus terrestris after anaerobiosis. Temp. 25°.*

Exp.	Wt. of worms g.	No. of worms	Period of anaerobiosis	Oxygen debt mm. ³	Recovery Excess oxygen used (mm. ³) hours recovery			
					$\frac{1}{2}$	1	$1\frac{1}{2}$	2
1	4.19	3	1 hr. 0 min.	450	251	411	418	419
2	3.84	2	1 10	360	176	269	314	314
3	3.31	4	1 10	177	150	172	182	198
4	4.09	2	1 0	329	274	373	388	376
5	3.86	2	1 0	278	258	353	—	—

There is a marked difference between the time taken for recovery in the cockroach and in the earthworm. Whereas in the earthworm the recovery is complete in most cases within a time equal to that of the period of anaerobiosis, in the cockroach the period is three times as long.

Lactic acid content. Lactic acid was estimated in freshly caught earthworms, in order to establish a resting control, and also after 1 hour's anaerobiosis at 25°. The results are given in Table III together with the calculated lactic acid content on the basis of the relationship in vertebrate muscle, *i.e.* 1 part of lactic acid oxidised for 4.4 removed. The average resting oxygen intake was assumed for purposes of this calculation to be 120 mm.³/g./hour, as the worms used weighed between 0.5 and 0.8 g.

Table III. *Lactic acid content of earthworms.*

Number of experiments	Time of anaerobiosis	Temp.	Lactic acid found mg. %	Mean deviation %	Excess lactic acid over resting		Ratio Found/Calc.
					Found mg. %	Calc. mg. %	
4	Control	—	41	9.0	—	—	—
5	1 hour	25°	122	12.5	81	72	1.1

DISCUSSION.

From the experimental results it seems reasonable to conclude that the oxidation process is merely deferred during anaerobiosis until oxygen is re-admitted, when the products of metabolism are removed by an excess of oxygen equal to that which would have been used had the normal metabolism continued undisturbed. These results are compatible with the breakdown of glycogen to lactic acid followed by the oxidative removal of the latter.

The results obtained in the estimation of the lactic acid confirm this view, in that the anaerobic life is accompanied by a considerable accumulation of this acid.

The relation between the oxygen debt and the excess of lactic acid, unlike the corresponding relationship in the cockroach, shows a close agreement with the conditions found in vertebrate tissue, the oxygen used in recovery being only about one-fifth of that necessary for the complete oxidation of the accumulated acid. It must be concluded therefore that this complete oxidation, if that be the true explanation of the results obtained on the cockroach, does not extend to the annelid worms, and further investigation will be necessary in order to define the biological boundaries within which the synthetic recovery process is not found. The result in the case of the earthworm agrees with its low oxygen intake as compared with the cockroach, and it would seem reasonable to search for other examples of complete oxidation amongst those animals whose oxygen intake is relatively high.

It is necessary to comment shortly on the earlier work of Lesser, and to try to explain the results he obtained in the light of the present investigation. Lesser's experimental findings were briefly as follows.

(1) During anaerobiosis glycogen is used in excess of the normal resting consumption.

(2) Carbon dioxide is evolved during anaerobiosis.

(3) The respiratory quotient is depressed during recovery.

(4) An excess of oxygen over the resting intake is used during recovery.

(5) Small quantities of a fatty acid are produced. This acid is volatile in steam and is probably valeric acid.

In explanation of these facts Lesser suggested that during anaerobiosis glycogen broke down to give carbon dioxide, the volatile fatty acid and another product which he failed to identify. Thus an excess of glycogen would be required during the anaerobic period, carbon dioxide would be evolved and the fatty acid would accumulate; following the re-admission of air, excess oxygen would be required to burn the fatty acid, and simultaneously the respiratory quotient would be lowered.

The facts, however, can be explained equally simply on the basis of the breakdown of glycogen to lactic acid, with the subsequent removal of the acid by oxidation and re-synthesis of glycogen. Thus, during the absence of oxygen, glycogen will be used without re-synthesis, and therefore, instead of the disappearance of only 1 part of each 4.4 parts degraded to lactic acid, the whole of the glycogen will be temporarily lost. The excess glycogen used during anaerobiosis will be 3.4 times as large as the normal consumption. If the value 100 mm.³/g./hour is assumed for the normal oxygen intake, it is possible to calculate the resting glycogen consumption in air, and hence the excess glycogen required in the absence of oxygen. This calculated value is given in column 4 of Table IV, whilst the corresponding experimental values are given in column 5. The agreement on the whole is good.

The accumulation of lactic acid during anaerobiosis will change the acid base equilibrium of the tissues with a resultant evolution of carbon dioxide. A corresponding change in the opposite direction will occur during recovery as the lactic acid is removed. These changes can account for the evolution of carbon dioxide during anaerobiosis and the lowered respiratory quotient during recovery. In column 6 of Table IV are given values for the calculated output of carbon dioxide during anaerobiosis, on the assumption that the excess of glycogen disappearing, as found by Lesser, is turned into lactic acid and neutralised by sodium hydrogen carbonate; in column 7 are the corresponding values for carbon dioxide evolved during anaerobiosis, as found by Lesser. It will be noted that there is a fairly uniform relationship between the values calculated and found, and that the calculated in all cases except Exp. XLI is larger than the observed. This is to be expected, as bases other than carbonates must take part in the buffering.

The increased oxygen consumption requires no comment. The presence of volatile fatty acids in quantities less than one-tenth of the excess of glycogen used can only be explained by the activity of bacteria in the intestinal tract of the worm, a similar explanation to that suggested in the case of the intestinal worm.

Table IV. *Recalculation of the experimental data given by Lesser.*

Experiment number (Lesser)	Weight of worms g.	Period of anaero- biosis hours	Excess glycogen		Carbon dioxide evolved during anaerobiosis	
			Calc. mg.	Found mg.	Calc. mg.	Found mg.
XXXVII	62.54	4	112	169	84	47
XXXVIII	98.20	5	221	163	81	72
XXXIX	119.04	4	212	208	104	76
XLI	74.00	5	167	49	24	55
XLIX	99.90	6	270	333	165	53

It may be assumed, therefore, that the data given by Lesser are not incompatible with the present conception of the source of anaerobic energy in the earthworm, and in view of the experimental evidence given above it seems reasonable to accept the breakdown of glycogen to lactic acid as the metabolic process involved in the maintenance of anaerobic life.

SUMMARY.

(1) The earthworm (*Lumbricus terrestris*) goes into debt for oxygen when it is kept under anaerobic conditions, using an excess volume of oxygen during recovery in air equal to that which it would have consumed during the anaerobic period.

(2) During the anaerobic period lactic acid accumulates in the tissues of the worm.

(3) The amount of lactic acid formed in a given time is related to the oxygen debt in the same way as in isolated vertebrate tissue. Thus in distinction to the cockroach there is a re-synthesis of glycogen.

(4) The data previously obtained by Lesser for the changes taking place in the earthworm during anaerobiosis can be explained by the breakdown of glycogen to lactic acid without the assumption of any special mechanism.

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